UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 3, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cancer Assessment Review Committee Meeting on Kresoxim-Methyl

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Sanjivani Diwan 3/3/99

TO: Addressees

Attached for your review is a package on Kresoxim-Methyl prepared by Myron S. Ottley.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday March 17,1999 at 10:00 am in Room 813, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
M. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

23-FEB-1999

MEMORANDUM

SUBJECT: KRESOXIM-METHYL (PC Code 129111): Evaluation of Carcinogenic Potential

FROM: Myron S. Ottley 3/1/99
Registration Action Branch I
Health Effects Division (7509C)

THROUGH: Melba Morrow, Branch Senior Scientist 3/1/99
Registration Action Branch I
Health Effects Division (7509C)

TO: William Burnam
Chairperson
Cancer Assessment Revbiew Committee
Health Effects Division (7506C)

Attached is the Cancer Assessment Document for kresoxim-methyl. The Cancer Review Assessment Committee is asked to review the database on kresoxim-methyl and render decisions on:

i. The carcinogenicity of the compound.

ii. Whether the submitted mode of action studies clearly demonstrate how liver tumors are formed in rats.

iii. Implication of the entire weight of evidence on the classification of kresoxim-methyl as a carcinogen, and its impact on human risk.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

KRESOXIM-METHYL

DRAFT REPORT

23-FEB-1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

3

DATA PRESENTATION: _____

DOCUMENT PREPARATION: _____

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

List the Committee members _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Consulting Pathologist

Statistical Analysis

OTHER ATTENDEES:

CONTENTS

Executive Summary iii

I. Introduction 1

II. Background Information 1

III. Evaluation of Carcinogenicity 1

1. Chronic Toxicity Study in Rats 1

2. Carcinogenicity Study in Rats 6

3. Carcinogenicity Study in Mice 9

2. Mode of Action Studies 12

IV. Toxicology 19

1. Metabolism 19

2. Mutagenicity 21

3. Structure Activity Relationship 22

4. Acute, subchronic and chronic Toxicity 22

V. Committee's Issues for Consideration 26

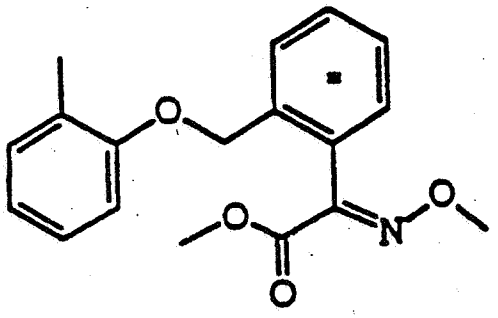
EXECUTIVE SUMMARY

7

I. INTRODUCTION

II. BACKGROUND INFORMATION

BASF Corporation has submitted a petition for the establishment of permanent tolerances for residues of kresoxim-methyl (PC Code 129111) in/on pome fruit, apple pomace, grapes and pecans. Kresoxim-methyl belongs to a new fungicide family, the strobilurins, which are synthetic analogs of strobilurin A. Strobilurin A is a naturally occurring antifungal metabolite a of pine cone mushroom *Strobilurus tenacellus*. The antifungal action is due to inhibition of mitochondrial respiration by blocking electron transfer in fungi



Kresoxim-methyl

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Chronic Toxicity Study with Kresoxim-methyl in Wistar Rats

Reference: Chronic Study of Kresoxim-methyl Administered in Feed to Wistar rats for 24 Months (1994). Study No. D67056, Laboratory Project No.. 70C0180/97007. dated 11/05/1994. **MRIDs** 43864246, 43864247

A. Experimental Design

Kresoxim-methyl (92.7 96.6% a.i.) was administered in the diet to Wistar rats (20/sex/dose) at dose levels of 0, 200, 800, 8000, or 16000 ppm (0, 9, 36, 370 and 746 mg/kg/day in males and 0, 12, 48, 503,and 985 mg/kg/day in females, respectively) for 24 months.

8

B. Discussion of Tumor Data

There was a dose-related increase in the incidence of hepatocellular carcinoma in both males and females at 8000 and 16,000 ppm (males: 0/20, 1/20, 1/20, 3/20, 8/20; females: 1/20, 0/20, 2/20, 6/20, 6/20 at 0, 200, 800, 8000, and 16,000 ppm, respectively. Although the increase was statistically significant only in 16,000 ppm males ($p \leq 0.01$), it was most likely treatment-induced in 8000 ppm females as well. This is supported by positive results in a oncogenicity study at the same doses and within the same strain of rats. The relationship to treatment of the sporadic liver cholangioma and/or cholangiocarcinoma (0 to 2 animals/group) in unknown.

Table 1a. Male Rats: Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

ppm	0	200	800	8000	16000
mg/kg/day	0	9	36	370	746
Tumor Type					
Adenoma	0/20	0/19	0/19	0/20	0/19
%	0	0	0	0	0
p =	-	-	-	-	-
Carcinoma[#]	0/20	1/19	1/19	3/20	8[a]/19
%	0	5	5	15	42
p =	-	0.487	0.487	0.115	0.001**

Table 1b. Female Rats: Hepatocellular Tumor Rates[+] and Peto's Test Results.

ppm	0	200	800	8000	16000
mg/kg/day	0	12	48	503	985
Tumor Type					
Adenoma	0/20	0/19	0/19	0/20	0/19
%	0	0	0	0	0
p =	-	-	-	-	-
Carcinoma[#]	1/20	0/19	2/19	6/20	6[a]/20
%	5	0	11	30	32
p =	0.001**	-	0.143	0.014*	0.014*

9

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First carcinoma observed at week 75, dose 16000 ppm.
[#]No adenomas observed.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Relevance of Tumor Type, Comparison with Historical Controls Significant treatment-related neoplastic findings were confined to the liver in both sexes of rats. There was a dose-related increase in the incidence of hepatocellular carcinoma in both males and females at 8000 and 16,000 ppm. It was statistically significant only in 16,000 ppm males ($p \leq 0.01$), but was most likely also treatment-induced in 8000 and 16,000 ppm females. This assertion is supported by historic controls provided by the performing laboratory (BASF Department of Toxicology) indicating that the incidence of carcinoma in the present study female control group (5%) was seven-fold higher than the average incidence in 29 studies (0.7%). As it was not possible to obtain an incidence < 5% in the present study (other than zero) due to sample size, a statistically significant increase in liver carcinoma might have been seen with a larger sample size or in a repeat experiment. Additionally, in a 2-year oncogenicity study performed using the same doses and strain of rats (MRID 43864249), an increase in liver carcinoma was seen in females at 8000 ppm. The relationship to treatment of the sporadically seen liver cholangioma and/or cholangio-carcinoma is unknown. Many other organs had various spontaneous neoplasms with an incidence comparable to that of controls. Although the overall number of primary neoplasms and tumor-bearing animals was comparable in control and treated groups, the total number of malignant tumors was increased somewhat in 8000 ppm females and in both sexes at 16,000 ppm (not statistically analyzed), possibly due to the liver neoplasms. Although it is possible that the animals could have tolerated a higher dose of test compound (intake of 746 mg/kg/day for males and 985 mg/kg/day for females at 16,000 ppm), the intake for females approximated the highest dose recommended for testing according to EPA guidelines (1000 mg/kg/day).

In a separate study (MRID 43864246), the effect of Reg. No. 242 009 on hepatic cell proliferation was investigated. In this experiment, 5 male rats/dose were given 0, 200, or 16,000 ppm (0, 15, 1140 mg/kg/day, respectively) in the feed for 3 weeks, during the last week also being given bromodeoxyuridine from subcutaneously implanted osmotic mini-pumps. No treatment-related effects were seen (body weight, food consumption, clinical observations, liver weight, gross or microscopic lesions) except for a statistically significant increase (2 to 3-fold) in cell proliferation in the hepatic lobules of the 16,000 ppm group. This result confirms the present study (MRID 43864247) in identifying the liver as the target organ for toxicity, and suggests that there is the potential for progression of liver lesions to a cancerous state beginning as early as three weeks on treatment in 16000 ppm male treatment group, and possibly females.

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the organs of both sexes of rats are presented in Tables 2a and 2b.

Table 2a Incidence of macroscopic changes found in rats given Kresoxim-methyl for two years[1]

Organ - lesion	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Liver - cyst - mass	0/20 1/20	1/20 1/20	0/20 1/20	3/20 4/20	6/20** 2/20
Kidneys - retraction	1/20	1/20	2/20	0/20	7/20*
Females					
Liver - cyst - mass	2/20 0/20	7/20 0/20	5/20 1/20	5/20 2/20	4/20 3/20
Glandular stomach - lesion	0/20	3/20	5/20*	1/20	2/20
Ovaries - cyst	2/20	3/20	4/20	7/20	8/20*

Data were taken from pages 977-986, MRID 43864247.
[1]The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *$p \leq 0.05$; **$p \leq 0.01$.

11

Table 2b. Microscoptic Non-Neoplastic Lesions in Wistar Rats Fed Kresoxim-methyl

Organ: lesion	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Liver:					
eosinophilic foci	0/20	1/20	0/20	6/20**	8/20**
mixed cell foci	0/20	0/20	2/20	4/20	5/20*
cellular hypertrophy	0/20	0/20	3/20	4/20	7/20**
Adrenal cortex:					
angiectasis	2/20	3/20	10/20**	14/20**	4/20
Females					
Liver:					
eosinophilic foci	1/20	0/20	0/20	0/20	1/20
mixed cell foci	0/20	0/20	0/20	0/20	2/20
cellular hypertrophy	1/20	1/20	0/20	1/20	8/20**
Ovaries:					
stromal hyperplasia	3/20	10/20*	4/20	8/20	8/20
Kidneys: tubular casts	2/20	1/20	2/20	6/20	10/20**
Mandibular lymph nodes:					
erythrophagocytosis	0/19	0/5	2/8	0/4	6/20*

Data were taken from pages 1002-1020, MRID 43864247.
[1]The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *$p \leq 0.05$; **$p \leq 0.01$.

The microscopic pathological results implicate the liver as a target organ in both sexes, consistent with the clinical enzymology and organ weight alterations. There was a dose-related increase in the incidence of liver cysts in 16,000 ppm males ($p \leq 0.01$) and minor increases in liver cysts and masses in both sexes at 8000 and 16,000 ppm ($p > 0.05$). These were correlated in males with an increased incidence of liver eosinophilic foci, mixed cell foci, and cellular hypertrophy at 8000 and/or 16,000 ppm and in females with cellular hypertrophy at 16,000 ppm ($p \leq 0.05$ or 0.01). The ovarian pathologies that were found (hyperplasia, $p \leq 0.05$ at 200 ppm; cysts, $p \leq 0.05$ at 16,000 ppm) were probably not toxicologically relevant because they were not correlated with neoplasia and were statistically significant at 40-fold different doses. Other pathological findings in males (kidney retraction, adrenal cortex angiectasis) and females (glandular stomach lesions, mandibular lymph node erythrophagocytosis, kidney tubular casts)

were of equivocal etiology and toxicological significance because they lacked other pathological correlates and/or were not clearly dose-related.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was judged to be adequate for assessing carcinogenicity because a NOAEL and LOAEL were both defined, and carcinogenicity was observed at the higher dose levels. The LOAEL for both male and female rats is 8000 ppm (about 370 mg/kg/day in males and 503 mg/kg/day in females) under the conditions of this study. This is based in males on the increase in SGGT levels, liver weight and histopathological changes, and in females on the roughly 10% lowered body weights and weight gains throughout much of the study. The NOEL for both sexes is 800 ppm, corresponding to about 36 mg/kg/day in males and 48 mg/kg/day in females. Therefore, the highest dose tested for each sex, 16000 ppm, is considered to be adequate to assess the carcinogenicity of Kresoxim-methyl. The High-dose level was not considered to be excessive because there was no effect observed on mortality, and the observed effects mentioned above were not severe.

2. Carcinogenicity Study in Rats

Reference: Oncogenicity Study of Kresoxim-methyl Administered in Feed to Wistar Rats for 24 Months. BASF Study No. D-67056; Project No. 70C0180/91006, dated 10/24/1994; **MRIDs** 43864246, 43864249.

A. Experimental Design

In an oncogenicity feeding study (MRID 43864249) Reg. No. 242 009 (92.7-96.6% w/w; Lot Nos. 91/180 (N27 IIIa1), 91/180-1 (N30 IIIa2), and 91/180-2 (N36 IIIc1) was administered to 50 Wistar rats/sex/dose in the diet for 24 months at dose levels of 0, 200, 800, 8000, or 16,000 ppm (mean compound intake in males was 0, 9, 36, 375, and 770 mg/kg/day and for females was 0, 12, 47, 497, and 1046 mg/kg/day, respectively).

B. Discussion of Tumor Data

Liver carcinoma was the primary neoplastic finding in both sexes of rats (Tables 3a and 3b), consistent with the histopathological findings. Its incidence was more remarkably increased in females than in males. The incidence in females was 1/50 (controls), 1/50, 2/50, 13/50, 16/50 ($p \leq 0.001$ at 8000, 16,000 ppm) and in males was 7/50 (controls), 5/50, 2/50, 18/50, 11/50 ($p \leq 0.05$ at 8000 ppm). Other liver neoplasms were found sporadically ($p > 0.05$) in one or both sexes (adenoma, hepatocholangiocarcinoma, cholangioma, hemangioma, hemangiosarcoma). The incidence and absolute number of all benign neoplasms were similar in treated and control groups (males & females). The overall incidence and absolute number of malignant neoplasms,

however, were remarkably increased in 8000 and 16,000 ppm females due to the increase in liver tumors.

Table 3a. Male Rats: Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

ppm	0	200	800	8000	16000
mg/kg/day	*0*	*9*	*36*	*375*	*770*
Tumor Type					
Adenoma	1/48	0/50	0/49	1[a]/50	0/47
%	2	0	0	2	0
p =	0.443	0.490	0.495	0.742	0.505
Carcinoma	7/48	5/50	2/49	18/50	11[b]/50
%	15	10	4	36	23
p =	0.002**	0.351	0.075	0.013	0.202
Combined	8/48	*5/50*	*2/49*	19/50	11/47
%	17	*10*	*4*	38	23
p =	0.002**	*0.250*	*0.042**	0.016*	0.287

[+]No. of tumor bearing animals/No. of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 98, dose 8000 ppm.
[b]First carcinoma observed at week 82, dose 16000 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3b. Female Rats: Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results.

ppm	0	200	800	8000	16000
mg/kg/day	0	12	47	497	1046
Tumor Type					
Adenoma	0/49	1/50	2/50	2*/50	1/50
%	0	2	4	4	2
p =	0.353	0.505	0.253	0.253	0.505

Carcinoma % p =	1/49 2 0.000**	1/50 2 0.748	2/50 4 0.508	*13[b]/50* *26* *0.001***	16/50 32 0.000**
Combined % p =	1/49 2 0.000**	2/50 4 0.508	4/50 8 0.188	15/50 30 0.000**	17/50 34 0.000**

[+]No. of tumor bearing animals/No. of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 107, dose 8000 ppm.
[b]First carcinoma observed at week 99, dose 8000 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the organs of both sexes of rats are presented in Tables 4a and 4b.

TABLE 4a: Incidence of macroscopic changes found in rats given Kresoxim-methyl for 2 years[1]

Organ-lesion	Dose (ppm)				
	0	**200**	**800**	**8000**	**16000**
Males					
Liver - cyst	3/50	2/50	7/50	5/50	9/50
- mass	3/50	5/50	3/50	10/50*	9/50
Testes - enlarged	4/50	8/50	13/50*	13/50*	9/50
Females					
Liver - cyst	5/50	7/50	6/50	7/50	12/50
- mass	0/50	0/50	2/50	7/50**	10/50**
Ovaries - cyst	5/50	8/50	7/50	11/50	12/50
- mass	0/50	1/50	3/50	4/50	6/50*

Data were taken from pages 468-480, MRID 43864249.
[1]The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *$p \leq 0.05$; **$p \leq 0.01$.

Table 4b. Incidence of microscopic findings in rats given Reg. No. 242 009 for 2 years[1]

Organ: lesion	Dose (ppm)				
	0	200	800	8000	16,000
Males					
Liver:					
eosinophilic foci	1/50	0/50	3/50	5/50	11/50**
mixed cell foci	4/50	1/50	2/50	9/50	12/50*
cellular hypertrophy	3/50	2/50	2/50	5/50	10/50*
biliary cyst(s)	1/50	4/50	5/50	7/50*	6/50
Females					
Liver:					
altered cell foci	27/50	34/50	32/50	34/50	39/50**
mixed cell foci	0/50	0/50	0/50	3/50	5/50*
bile duct prolifer	10/50	13/50	12/50	13/50	28/50***
cholangiofibrosis	1/50	4/50	1/50	5/50	7/50*
biliary cyst(s)	8/50	10/50	10/50	12/50	15/50
Ovaries: cyst(s)	19/50	23/50	35/50***	25/50	35/50***
Uterus/cervix: dilation	3/50	4/30	8/26**	4/27	13/50**
Brain: hemorrhage	1/59	2/50	3/50	4/50	7/50*

Data were taken from pages 602-621, MRID 43864249.
[1]The incidences were statistically analyzed by the reviewer using the Fisher exact test. Values significantly different from controls are designated: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

D. Adequacy of Dosing for Assessment of Carcinogenicity

A sufficiently high dose was achieved in this study in both sexes of rats based on their decreased body weights and body weight gains and on liver lesions. The High-dose level was not considered to be excessive because there was no effect observed on mortality, and the observed effects mentioned above were not severe.

3. Carcinogenicity Study in Mice

Reference: Eighteen-month Feeding/Carcinogenicity Study in Mice. BASF Corp. Study No. 94/10919, Project No 65C01810/91028, dated October 17, 1994; MRID 43864250.

A. Experimental Design

In a carcinogenicity toxicity study (MRID 43864250), kresoxim-methyl was administered to 50 C57BL/6N CrlBR mice/sex/dose in the diet at dose levels of 0, 400, 2000, and 8000 ppm for 18 months (approximate doses for males of 0, 60, 304, and 1305 mg/kg/day and for females of 0, 81, 400, and 1662 mg/kg/day). An additional 10 animals/sex/dose were treated for 12 months in a satellite study.

B. Discussion of Tumor Data

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Major organs examined included liver, thyroid, kidney, testes, ovary, bladder, lung, and brain. The incidence and nature of tumors observed are presented in Table 5 below:

TABLE 5: Number of animals with neoplastic lesions in The 18 month study with Kresoxim-methyl

Organ/Lesion	Lesion incidence/50 animals							
	Control		400 ppm		2000 ppm		8000 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver/ Hepatocellular Carcinoma	4	1	3	1	0	0	3	0
Liver/ Hepatocellular Adenoma	0	0	1	0	1	0	1	1
Lung/ Adenoma	2	0	2	1	1	2	3	2
Lung/ Adenocarcinoma	0	0	0	0	0	0	1	0

Data extracted from Pathology Report, pp. 0484-0485, MRID 43864250.

C. Non-Neoplastic Lesions

Few lesions were seen that were higher in any treated groups than in the control groups and none showed a dose-response relationship (Tables 6a and 6b). The incidence of kidney retraction was increased in high dose females, and a slight, but not significant, increased incidence of enlarged spleen was seen in high dose females. An increased incidence of enlarged spleen was also seen

in females that died prior to study termination (2/10, control; 1/6, 400 ppm; 3/9, 2000 ppm; and 5/8, 8000 ppm).

TABLE 6a: Number of animals with gross lesions in the 18-month study with Kresoxim-methyl

Organ/Lesion	Lesion incidence/50 animals							
	Control		400 ppm		2000 ppm		8000 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Kidney/Retraction	2	4	1	0	4	2	1	13*
Spleen/Enlarged	6	9	7	6	3	9	2	11
Liver/Enlarged	2	1	2	1	1	3	0	3
Liver/Focus	4	5	4	2	3	0	2	7

Data extracted from Pathology Report, pp. 0454-0458, MRID 43864250.
*$p \leq 0.05$ Significantly different from controls. Fisher exact test performed by reviewer.

Non-neoplastic - Selected microscopic findings that may be treatment-related are shown in Table 5b. The incidence of papillary necrosis of the kidney was increased in females at 8000 ppm. The incidence of amyloidosis in the liver of both sexes was increased at 8000 ppm, and was increased in the adrenal cortex of males at 8000 ppm. The severity of liver amyloidosis in female mice was greater at 8000 ppm than at other doses or the control animals; the severity in males, however, at 8000 ppm was comparable to the control group. Dose-related effects were not seen with either lesion.

TABLE 6b Number of animals with microscopic lesions in the 18 month study with Kresoxim-methyl

Organ/Lesion	Lesion incidence/50 animals							
	Control		400 ppm		2000 ppm		8000 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
Kidney/ Papillary necrosis	1	2	2	4	3	4	1	13**
Liver/ Amyloidosis	13	6	11	1	7	4	20	16*
Adrenal cortex/ Amyloidosis	23	33	1/6[a]	2/6	6/8	3/9	34*	33

18

Liver/ Foci of cellular alteration	3	1	3	1	3	1	7	2

Data extracted from Pathology Report, pp. 0469-0474, MRID 43864250.
[a]Incidence/number of animals examined if less than 50.
*p≤0.05, **p≤0.01 Significantly different from controls. Fisher exact test performed by reviewer.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on decreased weight gain in females at 2000 and 8000 ppm, and in males at 8000 ppm, on kidney papillary necrosis in females at 8000 ppm, on liver amyloidosis in both sexes at 8000 ppm, and on adrenal cortex amyloidosis in males at 8000 ppm. The high dose rate was above the limit dose of 1000 mg/kg/day for both sexes.

4. Mode of Action Studies

The Registrant has proposed that hepatocellular tumor induction in the rat by kresoxim-methyl is a result of clonal expansion of spontaneously initiated cells due to enhanced hepatocellular proliferation at high dose levels. Therefore, Kresoxim-methyl is not an tumorigenic initiator, it is just a promoter.

1. S-phase response studies:

a. In an S-phase response study (MRID 44380201) kresoxim-methyl (tech., 94.3% a.i.) was administered for 3 weeks to 5, 16-month old male Wistar rats/dose in the diet at dose levels of 0, 200 or 16,000 ppm (equivalent to an estimated average daily intake of 0, 10 or 800 mg/kg/day). Osmotic mini-pumps providing continuous delivery of bromodeoxyuridine (BrdU) at 20 mg/ml in normal saline were implanted at the end of week 2. Delivery of BrdU was continued until necropsy at the end of week 3. BrdU was localized in hepatocytes using immunohistochemical staining and the labeling indices of the periportal zone 1 in the 3 liver lobes of each rat were determined.

At 16,000 ppm, hepatocyte labeling indices of all 3 liver lobes were increased relative to controls by approximately 2.2 to 3.3-fold (mean 2.9-fold higher). There were no biologically significant differences in the labeling indices of the 3 lobes. Kresoxim-methyl did not cause increased labeling indices at 200 ppm. A comparison of labeling indices from this study with an earlier study conducted on 2 month old male rats at the same dose levels (described in this study report) demonstrated that similar increases relative to controls were observed in younger and older rats, although some differences in labeling indices of the individual liver lobes were observed. There were no treatment-related effects observed on body weight, liver weight or liver pathology. Food consumption was not determined.

This s-phase response study is classified Acceptable-non-guideline. The study was submitted as additional mechanistic data for kresoxim-methyl and does not satisfy a guideline requirement. However, it was adequately conducted and **demonstrated (when considered together with 2 other studies) that hepatocellular proliferation was increased in male rats at dietary levels at which increased incidence of hepatocellular neoplasms was also observed**. Some confirmatory data are requested for this study (see Discussion/Study Deficiencies section of DER for details).

b. In an S-phase response study (MRID 44392001) kresoxim-methyl (tech., 94.9% a.i.) was administered for 3 weeks to 5, 2-month old male Wistar rats/dose in the diet at dose levels of 0, 800 or 8,000 ppm (equivalent to an average daily intake of 0, 61 or 603 mg/kg/day). Osmotic mini-pumps providing continuous delivery of bromodeoxyuridine (BrdU) at 20 mg/ml in normal saline were implanted at the end of week 2. Delivery of BrdU was continued until necropsy at the end of week 3. BrdU was localized in hepatocytes using immunohistochemical staining. The labeling indices of the periportal zone 1 and the combined indices of periportal zone 1 and intermediate zone 2 in the 3 liver lobes of each animal was determined.

At 8,000 ppm, labeling indices of all 3 liver lobes were increased relative to controls by about 1.7- to 1.9-fold (mean 1.8-fold). Indices were highest in *lobus dexter medialis*, followed by *lobus dexter lateralis* and *processus caudatus*. No increase was observed at 800 ppm. The labeling indices for combined zones 1 and 2 hepatocytes were significantly increased in *l. dexter medialis* and *lateralis* (about 1.5 and 1.2-fold above controls, respectively). Although a significant increase was not observed in *p. caudatus*, the index was still about 01.5-fold higher than controls. All increases were lower than zone 1 alone, indicating that increased proliferation in response to treatment was largely localized to the periportal zone 1. There were no treatment-related effects observed on body weight, food consumption, liver weight or liver pathology.

This s-phase response study is classified Acceptable-non-guideline. The study was submitted as additional mechanistic data for kresoxim-methyl and does not satisfy a guideline requirement. However, it was adequately conducted and **demonstrated (when considered together with 2 other studies) that hepatocellular proliferation was increased at dose levels at which an increased incidence of hepatocellular neoplasms was also observed.**

2. *In vitro* metabolism study: In a special (non-guideline) metabolism study (MRID 44421001), *in vitro* assays were used to characterize and compare metabolism of BAS 490 F (tech., 97.2% a.i. and 99.2% radiochemically pure; label on the phenyl ring) in rats vs. mice. Males and females of both species were evaluated in the following assays (1) kinetics of ester bond cleavage in plasma, in which ^{14}C-BAS 490 F was incubated with plasma and the rate of the methyl ester cleavage determined by HPLC from samples taken during a 3-hr incubation and (2) evaluation of metabolite profiles by HPLC following a 6 hr incubation of isolated hepatocytes with ^{14}C-BAS 490 F.

There were no major differences observed between rats and mice in the rate of ester bond cleavage I plasma. Cleavage of BAS 490 F (as determined by the remaining parent compound

vs. 490 M1, a major metabolite) was complete by 15 minutes in male rats and mice and female mice and by 30 minutes in female rats. HPLC metabolite profiles from the isolated hepatocyte preparations were similar but not identical between rats and mice. Seven major peaks were separated in male rats (five in females). The same peaks were isolated in male mice and female mice (except for H1 in females), plus an additional peak, UK, tentatively identified as 490 M24 (also observed in the previously conducted *in vivo* rat metabolism study). Although the profiles indicated some quantitative differences between male and female rats and mice, no data on quantitation of the peaks was provided other than the HPLC profiles.

This special *in vitro* metabolism study is classified Nonguideline-Unacceptable. Although the report **provides some useful comparative information regarding the metabolism of Kresoxim-methyl in male and female rats and mice and suggests that both share similar pathways**, there were several deficiencies noted: some information was not included in the report: for example, (1) the number of animals used for each part of the study was not clearly indicated; (2) it was not clear why results of the HPLC elution system no. 3 were not mentioned (profiles from one female rat in systems 2 and 3 were provided) and (3) the raw LSC data for all hepatocyte suspensions were not included except for 1 female rat. In the isolated hepatocyte study, (1) no quantitation was performed on the peaks of the metabolite profiles and (2) only some of the metabolite peaks were tentatively identified and (3) this method does not appear to be sensitive enough to fully characterize the metabolites of BAS 490 F, since more metabolites were found in the *in vivo* rat metabolism study. This study was not submitted to satisfy the guideline requirement for a metabolism study (85-1) in the rat, but was submitted as additional information. Additional information to upgrade this study is not required at this time and will only be requested if the Agency determines that it is needed to better characterize the comparative metabolism of BAS 490 F for evaluation of mechanism of action.

PROPOSED MECHANISM OF ACTION FOR LIVER TUMORIGENESIS IN RATS

The Registrant has submitted a report summarizing the mechanism of action they propose to account for increased hepatocellular tumor formation observed at high dose levels in the rat (MRID 44341012)[1] and two documents providing justification for the use of BrdU labeling to evaluate proliferative response in hepatocyte, one prepared by BASF (MRID 44380202)[2] and the

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[1]Van Ravenzwaay. Toxicology Report, Kresoxim-Methyl: Mechanism and Assessment of Liver Tumor Induction. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/Rhine, FRG. BASF Registration Doc. No. 96/10078, February 12, 1996. MRID 44341012.

[2]Van Ravenzwaay, B. Toxicology Report: Statement Concerning the Determination of Cell Proliferation in the Liver of BAS 490 F Treated Rats. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/Rhine, FRG. BASF Registration Doc. No. 97/10286. March 31, 1997. MRID 44380202.

other by Drs. James Swenberg and Thomas Goldsworthy, experts in the use of methods for detection of cell proliferation (MRID 44380203)[3].

The rationale for the BrdU S-phase response labeling protocol used to evaluate hepatocellular proliferation was discussed. TB-I agreed that this is the most appropriate method for evaluating kresoxim-methyl. BrdU incorporation was selected over an alternative method for visualizing proliferating cells, PCNA (proliferating cell nuclear antigen) staining. Although PCNA immunohistochemical staining can be used to evaluate tissue slides from a previously conducted study, it has the disadvantages that (1) sensitivity is lower than BrdU incorporation, (2) cells in G1 and G2 phase as well as S phase will stain, making interpretation of the data more complicated and (3) only cells that are proliferating at the time of sacrifice are labeled. In S-phase response studies, BrdU must be introduced into animals to measure cell proliferation in the animals at that time. However, only cells in S-phase are labeled and by providing continuous administration of BrdU over a 1 week period (instead of a pulse administration), the number of labeled cells, and therefore sensitivity of the assay, can be increased. The sensitivity of the assay is important for evaluation of cellular proliferation for kresoxim-methyl because the response appears to be localized to the periportal zone 1 of the liver lobule. In their expert report, Drs. Swenberg and Goldsworthy concurred with the selection of this method.

The Registrant has proposed that hepatocellular tumor induction in the rat by kresoxim-methyl is a result of clonal expansion of spontaneously initiated cells due to enhanced hepatocellular proliferation at high dose levels. The data pertinent to evaluation of mechanism of action of kresoxim-methyl are summarized below:

1. In the 2-year rat dietary chronic toxicity/carcinogenicity study (MRID 43864249), an increased incidence of hepatocellular carcinomas was observed in males and females at 8000 and 16000 ppm. Dose groups and tumor incidence are summarized below in Table 7.

Table 7: Incidence of Hepatocellular Carcinoma in Rats

	Dietary concentration in ppm				
Sex of animals	0	200	800	8,000	16,000
Males: Dose, mg/kg/day	0	9	36	375	770
Hepatocellular carcinoma Percent incidence	7 14%	5 10%	2 4%	18* 36%	11 22%

[3]Swenberg, J. And Goldsworthy, T. Toxicology Report: Expert Report on the Evaluation of Hepatic Cell Proliferation in Liver from Rats Exposed to BAS 490 F. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/Rhine, FRG. BASF Reg. Doc. No. 96/11092. December 9, 1996. MRID 44380203.

Females: dose, mg/kg/day	0	12	47	497	1,046
Hepatocellular carcinoma Percent incidence	1 2%	1 2%	2 4%	13* 26%	16* 32%

* Statistically significant, $p \leq 0.05$. N = 50, all groups.

2. Other signs of toxicity at 16,000 ppm included decreased body weights, hepatocellular hypertrophy and increased liver weights, increased bile duct proliferation in females and increased incidence and severity of eosinophilic foci of hepatocellular alteration. Decreased body weights were observed at 8,000 ppm. The NOAEL was 800 ppm.

3. Kresoxim-methyl did not show genotoxic potential in the following assays (MRIDs 43864254 to -61): Ames (two studies), Chinese hamster ovary/HPRT point mutation, human lymphocyte *in vitro* cytogenetics, mouse *in vivo* micronucleus, rat hepatocyte unscheduled DNA synthesis (two *in vivo* and one *in vitro* tests).

4. In a special study to evaluate the initiation potential of kresoxim-methyl (Gamer *et al.*, 1995, BASF Reg. # 95/10567; not submitted for review), there was no treatment-related induction of glutathione-s-transferase P (GST-P)-positive (initiated) cell foci in the liver following a single gavage dose of kresoxim methyl. Ten Wistar rats/sex were assigned to the following groups: positive controls, administered 28 mg/kg N-nitroso-morpholine (NNM); negative controls, administered 0.5% carboxymethylcellulose vehicle only and the treatment group, administered 2,388 mg/kg kresoxim-methyl. A partial hepatectomy was performed on all animals and 14 hrs later, a single gavage dose of kresoxim methyl, NNM or vehicle only was administered. After a 14-day recovery period, half of the animals in each group were administered 500 ppm phenobarbitone in the diet for 8 weeks (to promote cell proliferation of initiated foci and thereby increase assay sensitivity) and half were maintained untreated. After sacrifice, microscopic evaluation of liver tissue was performed following hematoxylin and eosin staining and also staining for GST-P.

   There were no increases in the number of initiated foci in kresoxim-methyl treated rats. The number of GST-P positive foci per liver was 0-3 in both kresoxim-methyl groups, which was within the negative control values. In contrast, the positive controls had 3-100 foci/liver (no phenobarbitone) and 5 - >100 foci with phenobarbitone treatment. GST-P positive foci/cm^2 of liver tissue were lower in the kresoxim-methyl groups (0.12 and 0.18) than the controls (0.20 and 0.30).

5. Because kresoxim-methyl did not show initiating potential, based on negative results in mutagenicity studies and lack of induction of GST-P positive foci in the rat liver, studies to investigate how it acts as a promoter in the development of liver tumors were performed. The ability of kresoxim-methyl to increase the rate of cellular proliferation was evaluated in a ^{3}H-thymidine incorporation study (Polloth *et al.*, 1994, BASF Reg. # 94 10867; not submitted for review). Three male Wistar rats/dose group were administered single gavage doses of kresoxim-methyl at 0, 20, 200 or 1,000 mg/kg in

0.5% aqueous carboxymethylcellulose. Autoradiography of liver sections demonstrated an increase in the percent of S-phase cells at 200 and 1,000 mg/kg (2.8% and 2.6%, respectively vs. 1% and 1.4%, at 0 and 20 mg/kg, respectively).

6. S-phase response studies were conducted to further characterize the proliferative response. Two of these studies were submitted for review and Executive Summaries are provided above (a third study was described in one of these reports). The studies demonstrated that kresoxim-methyl caused an increase in hepatocellular proliferation at dose levels that also resulted in increased incidence of hepatocellular carcinoma (8,000 and 16,000 ppm). The increase was localized to the periportal zone 1 of the liver lobule, was observed in all liver lobes, and response was similar in older (15-month) and younger (2-month) male rats. The mean labeling indices observed at each dose level (in 2-month old rats) are summarized below in Table 8:

Table 8; Relative labeling indices in zone 1

Dose (ppm)	0	200	800	8,000	16,000
Labeling indices relative to controls (100%)	100%	125%	96%	180%*	311%*

7. In addition to the submitted S-phase response studies, two other studies conducted by BASF were summarized in the Toxicology Report document. In one study (Mellert *et al.*, 1996, BASF Reg. # 96/10053; not submitted for review), ten groups of 5 male Wistar rats were administered kresoxim-methyl at 0 or 16,000 ppm in the following protocol (Table 9):

Table 9: Protocol for s-phase response study #96/10053

Test group	Concentration in diet, ppm	Treatment period, weeks	Recovery period, weeks
0	0	1	-
1	16,000	1	-
2	0	1	2
3	16,000	1	2
4	0	6	-
5	16,000	6	-
6	0	13	-
7	16,000	13	-
8	0	13	5
9	16,000	13	5

Osmotic mini-pumps were implanted one week before sacrifice for continuous delivery of BrdU. Results for test groups without recovery periods are summarized below in Table 10:

Table 10: Labeling indices as a percent of controls following exposure to kresoxim-methyl (#96/10053)

	Relative Labeling Index			
Test Group	Zone 1	Zone 2	Zone 3	Mean
Controls	100	100	100	100
1 week exposure	175*	170*	162	171*
6 weeks exposure	216*	92	95	149
13 weeks exposure	144	139	93	137

Animals that were sacrificed after a 2 or 5 week recovery period showed a decrease in cell proliferation. Labeling indices were 40% of control levels in animals treated for 1 week and 30% of control levels in animals treated for 13 weeks.

From the above data, the Registrant concluded the following:

- Based on lack of genotoxic potential and negative results in the GST-P positive foci assay, it was concluded that kresoxim-methyl is not an initiator of liver carcinogenesis.

- By default, kresoxim-methyl was determined to be a promoter of liver carcinogenesis. In the BrdU S-phase incorporation studies, increased hepatocellular proliferation was observed at the same dietary dose levels at which increased incidence of hepatocellular carcinomas were observed. This effect was localized in the periportal zone 1 (after a transient initial increase in all zones). The response did not appear to be affected by the age of the animal.

- Additional data are consistent with promoter activity. Increased hepatocellular proliferation was reversible in an S-phase study with recovery periods following dosing at 16,000 ppm. Long-term administration at relatively high dose levels was required for liver tumor formation in the dietary studies and tumors did not cause mortality. No tumors were observed in organs other than the liver.

- It is therefore proposed that with long-term exposure at high dose levels, kresoxim-methyl causes clonal expansion of spontaneously initiated hepatocytes in the periportal zone 1 of the liver lobule and thereby increases the incidence of hepatocellular tumors. It is not clear at this time whether the increased proliferation is secondary to hepatocellular toxicity or occurs by some other mechanism, although liver GGT levels have been shown to increase at high doses, which is suggestive of liver damage. Because the proliferative effect appears to have a threshold, increases in hepatocellular tumors above background would also be expected to have a threshold.

The data provided by the Registrant are consistent with increased cellular proliferation as a mechanism of increased liver tumor formation. In their Expert Report, Drs. Swenberg and Goldsworthy state that: "The cell proliferation studies completed to date on BAS 490 F strongly suggest that sustained increases in hepatocyte proliferation from the periportal regions of livers from rats exposed to 8,000 or 16,000 ppm BAS 490 F are an important mechanism related to the induction of liver neoplasms by this chemical". However, the experiments performed did not fully evaluate the time course of this increase, the effect of prolonged dosing (beyond 13 weeks) or whether a maximum dose effect is reached at any dose level. The time course S-phase response study suggests a decrease in the proliferative effect with increasing exposure duration and also demonstrated recovery. It is also not known whether the tumors arise primarily in the periportal zone 1 where increased cellular proliferation is sustained. Tumorigenic and cellular proliferative effects at dose levels between 800 and 8,000 ppm have not been evaluated. Although the studies are suggestive of increased cellular proliferation as a factor in development of hepatocellular tumors, other potential mechanisms of tumorigenesis (e.g., decreased apoptosis) are not necessarily ruled out.

It is not known at this time whether the increase in liver tumors is a species-specific response unique to the rat. S-phase response studies were not performed in mice, which did not develop liver tumors in response to long-term dietary exposure to doses exceeding 1,000 mg/kg/day. The *in vitro* studies comparing rat and mouse metabolism of BAS 490 F indicated similar, but not identical, metabolic profiles. However, the study did not provide a full characterization of metabolism in these two species and there may be differences observed *in vivo* that were not detected in the isolated hepatocyte incubation assays.

IV. TOXICOLOGY

1. Metabolism

In a metabolism study, kresoxim-methyl with [^{14}C]-label on ring A (Batch No. 358-01) or ring B , was administered to Wistar rats (2-12/sex/group depending on the experiment) as single gavage doses of 50 or 500 mg/kg or 15-day repeated doses of 50 mg/kg, or as a single intravenous dose of 5 mg/kg/day. [^{13}C]-Labeled test compound was included in one 500 mg/kg dose group to facilitate metabolite identification. Biliary metabolites were assessed in rats with cannulated bile ducts given an oral dose of 50 or 500 mg/kg kresoxim-methyl

No animals died as a result of the treatment. The overall recovery of radiolabeled compound was acceptable, ranging from 87.38-101.24%. Orally administered test compound was widely distributed and quickly eliminated; results indicated there was no bioaccumulation. In both sexes, the major routes of excretion were feces (66-81% of given dose) and the urine (9-33% of given dose). No radioactivity was detected in exhaled air.

Based on urinary excretion of about 20-28% and biliary excretion of about 35-43% of the given dose (separate experiments), about 63% of the given radioactivity was absorbed 48 hours after a

single 50 mg/kg oral dose in both sexes. At 500 mg/kg, however, rats excreted only about 8-13% of the given dose in the urine and 14-15% in the bile (total of about 23-27% of dose) after 48 hours. This was due to absorption saturation; there was no evidence for an increased tissue burden. Consistent with absorption saturation, relatively more radioactivity was in the feces of high-dose rats, whereas intravenously dosed rats (5 mg/kg) excreted relatively more radiolabel in the urine and less in the feces. Parent compound accounted for up to 74.9% of administered radioactivity in feces of orally dosed rats but was not detected in the urine. It was present, however, in the urine (16.3% of given dose) and feces (7.7% of given dose) of the intravenously dosed females; the latter was inconsistent with the lack of parent compound in the bile of orally dosed rats. Absorption saturation was also seen in the area under the curve for plasma radioactivity vs. time, which was only about 2-fold greater at the high than at the low dose.

A total of 32 different metabolites were identified in the urine, feces, bile, plasma, liver, and kidneys of rats given Reg. No. 242 009. There were some sex, dose, route, and label-dependent differences in the metabolite profiles. M9 or M1 were the most abundant metabolites in the urine and feces of all groups. Biliary metabolites included M1 and M9 as well as several glucuronide metabolites not present in urine or feces. Characterization of biliary metabolites from low-dose rats appeared to be inadequate due to a 60% deficiency in recovered radioactivity. Metabolite profiles of the plasma, liver, and kidneys were similar, the predominant metabolites being M1 and M9. In the proposed metabolic pathway, ester cleavage of a methyl group is proposed to be the most important initial reaction, forming M1, which is then hydroxylated to form M9 or M2, and finally conjugated by glucuronic acid or sulphate (MRIDs 43864262, 43864263, 43864264, 43883604, and 43883605).

2. <u>Mutagenicity</u>:

The data base for Mutagenicity is considered adequate. The studies were considered acceptable and met the Subdivision F Guideline requirements for mutagenicity testing. Based on the available mutagenicity studies, there are no concerns for mutagenicity at this time.

(i) In an Ames assay, when tested in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 at concentrations up to 5000 µg/plate, Kresoxim-methyl was non mutagenic with or without metabolic activation (MRID 43864254, 43864258).

(ii) In a mammalian cell forward gene mutation assay at the HPRT locus,,when tested at concentrations up to 100 µg/mL in the presence and absence of S9-mix , Kresoxim-methyl did not induce mutant colonies over background. (MRID 43864255).

(iii) In a mammalian cell cytogenetics assay (chromosomal aberrations), primary human lymphocyte cultures from healthy volunteers were exposed to Kresoxim-methyl in acetone at concentrations up to 40 µg/mL with and without metabolic activation (S9-mix). There was no evidence that Kresoxim-methyl induced chromosomal aberrations (structural or numerical) over background, with or without S9-mix, at any concentration tested (MRID 43864256).

(iv) In an NMRI mouse bone marrow micronucleus assay at doses up to 2000 mg/kg body weight., Kresoxim-methyl produced no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose of Reg. No. 242 009 tested in this study (MRID 43864257).

(v) In an unscheduled DNA synthesis assay primary rat hepatocyte cultures were exposed to kresoxim-methyl at concentrations up to 100 µg/mL for 18 hours. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced by the test material (MRID 43864259).

(vi) In an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay, male Wistar rats were exposed to Kresoxim-methyl in their diet at 200 or 16,000 ppm for three weeks Hepatocytes were then isolated and the UDS determination made in the cultured cells. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced by the test article (MRID 43864260).

(vii) In an *ex vivo* unscheduled DNA synthesis (UDS)assay, male Wistar rats were given a single oral dose (gavage) of Kresoxim-methyl at concentrations up to 1000 mg/kg body weight. Approximately 18 hours after treatment hepatocytes were isolated and analyzed in culture for induced UDS activity. The percentage of hepatocytes in S-phase was also determined. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced by Kresoxim-methyl as tested in this study; however, an increase (>2.5x) in the mean percentage of S-phase cells over solvent control values was seen at 200 and 1000 mg/kg body weight treatment. This approached, but did not meet, the laboratory's criterion (≥3.0x increase) for a positive S-phase response (MRID 43864261).

3. Structure-Activity Relationship

There are no chemicals structurally related to kresoxim-methyl in the HED database that have been found to be carcinogenic. Therefore structure-activity relationship has not been examined.

4. Acute, Subchronic, and Chronic Toxicity

a) Acute Toxicity

Kresoxim-methyl is not acutely toxic via the oral, dermal or inhalation routes of exposure (Toxicity Categories all IV), is not skin irritant (Toxicity Category IV), is a mild eye irritant (Toxicity. Category III). In an acute neurotoxicity study in rats, the NOAEL was 2000 mg/kg (the Limit Dose, and the LOAEL was not established.

b) Subchronic Toxicity

82-1a Subchronic Oral Toxicity Feeding - Rat

In a subchronic toxicity study (MRID 43864245) Reg. No. 242 009 (98.7% w/w, Lot #N 21) was administered to 10 Wistar rats/sex/dose in the diet at dose levels of 0, 500, 2000, 8000, or 16,000 ppm (0, 36, 146, 577, 1170 mg/kg/day for males and 0, 43, 172, 672, 1374 mg/kg/day for females, respectively) for 3 months.

All the animals survived to study termination. There were minor, statistically significant though not dose-related decreases in the body weights (≤ 9.5%, $p \leq 0.05$ or 0.01) and body weight gains (≤ 15.2%, $p \leq 0.05$) in 8000 and 16,000 ppm males. These were not accompanied by effects on food consumption or food efficiency. Gamma-glutamyl transferase (GGT) was elevated in males in a time and dose-dependent manner, reaching statistical significance at 8000 and 16,000 ppm on day 89 (about a 5-fold increase, $p \leq 0.01$). The only histopathological finding was a minor change in the amount and/or distribution of fat in hepatocytes (all dose groups, both sexes); it is unclear whether this effect is treatment-related or toxicologically relevant. The relative liver weight was increased in males at 16,000 ppm and in females at ≥ 2000 ppm (7.3-11.9%, $p \leq 0.05$ or 0.01). These liver weight increases were not considered toxicological significant.

Under the conditions of this study, the LOAEL for male rats is 8000 ppm (577 mg/kg/day) based on elevated serum GGT (consistent with results seen in other studies); a LOAEL was not established in females. The NOAEL for males is 2000 ppm (146 mg/kg/day) and for females is 16000 ppm (1374 mg/kg/day.)

This subchronic toxicity study is classified as acceptable, and satisfies the guideline requirement for a subchronic oral study (82-1(a)) in rats.

82-1a Subchronic Oral Toxicity - Mouse

In a subchronic toxicity study (MRID 43864244) Reg. No. 242 009 (kresoxim-methyl)(98.7%a.i., Lot #N 21) was administered to 10 C57BL/6N CrlBR mice/sex/dose in the diet at levels of 0, 250, 1000, 4000, or 8000 ppm. These concentrations resulted in approximate dose levels of 0, 57, 230, 909, and 1937 mg/kg/day for males; and 0, 80, 326, 1326, and 2583 mg/kg/day for females.

The relative liver weights were significantly ($p \leq 0.01$) increased in males at 4000 (10.7%) and 8000 ppm (18.9%) compared to the controls. No gross or microscopic lesions were found that correlated with the increased relative liver weights. There were no neoplastic lesions found in treated animals. **A LOAEL was not determined for males and females. The NOAEL is 8000 ppm (2583 mg/kg/day for females and 1937 mg/kg/day for males).**

This subchronic toxicity study is classified as acceptable, and does satisfy the guideline requirement for a subchronic oral study (82-1) in mice. A LOAEL was not determined for males and females in the study, however, the high dose (1937 mg/kg/day-males; 2583 mg/kg/day-females) is higher than the limit dose of 1000 mg/kg/day.

c) Chronic Toxicity

83-1a Chronic Oral Feeding Toxicity - Rat

In a chronic toxicity study (MRID 43864247) Reg. No. 242 009 (92.7-96.6% w/w, Lots No. N 27 (IIIa1), N 30 (IIIa2), N 36 (IIIc1)) was administered to 20 Wistar rats/sex/dose in the diet at dose levels of 0, 200, 800, 8000, or 16,000 ppm (0, 9, 36, 370, and 746 mg/kg/day for males and 0, 12, 48, 503, 985 mg/kg/day for females, respectively) for 24 months.

There was no effect on mortality in either sex of rats. The body weights of 8000 and 16,000 ppm females were decreased throughout the study (about 3-10%; $p \leq 0.05$ or 0.01). Body weight gains paralleled body weight changes in both sexes; the changes were of minor biological significance. The level of serum gamma-glutamyl transferase (SGGT) was elevated 4.8 to 46-fold in 8000 and 16,000 ppm males throughout the study ($p \leq 0.01$; time and dose-dependent). Organ weight changes in 8000 and 16,000 ppm males included a dose-related increase in absolute and/or relative liver weight (16-22%, $p \leq 0.05$ or 0.01). The liver weight changes were correlated with the increased SGGT level in males and with numerous macroscopic and microscopic pathologies in both sexes, implicating the liver as a target organ.

The LOAEL for both male and female rats is 8000 ppm (about 370 mg/kg/day in males and 503 mg/kg/day in females) under the conditions of this study. This is based in males on the increase in SGGT levels, liver weight and histopathological changes, and in females on the roughly 10% lowered body weights and weight gains throughout much of the study. The NOAEL for both sexes is 800 ppm, corresponding to about 36 mg/kg/day in males and 48 mg/kg/day in females.

This chronic toxicity study is classified as acceptable, and satisfies the guideline requirement for a chronic oral study (83-1a) and carcinogenicity study (83-2a) in rats.

83-1b Chronic Oral Toxicity [capsule] - Dog

In a chronic toxicity study (MRID 43864248), kresoxim-methyl (93.7% a.i., Lot #91/180-2/N 36 (=III c 1)) was administered to 5 beagle dogs/sex/dose in the diet at levels of 0, 1000, 5000, or 25,000 ppm for 12 months. These concentrations resulted in approximate dose levels of 0, 27, 138, or 714 mg/kg/day for males and 0, 30, 146, or 761 mg/kg/day for females.

The mean body weight of the males in the 25,000 ppm group was significantly ($p \leq 0.02$) decreased from day 189 through the end of the study and was decreased approximately 11% at study termination. Body weight gain was decreased 57% in the 25,000 ppm group. A treatment-related decrease in food efficiency was also observed in males of the 25,000 ppm group. No other treatment-related effects were observed. **The LOAEL is 25,000 ppm (714 mg/kg/day) for males based on decreased mean body weight and body weight gain and decreased food efficiency. A LOAEL was not identified for females. The NOAEL is 5000 ppm (138 mg/kg/day) for males and 25,000 ppm (761 mg/kg/day) for females.**

This chronic toxicity study is classified as **acceptable** (83-1b). It satisfies the guideline requirement for a chronic oral study (83-1b) in dogs.

83-2a Carcinogenicity [feeding] - Rat

In an oncogenicity feeding study (MRID 43864249) Reg. No. 242 009 (92.7-96.6% w/w; Lot Nos. 91/180 (N27 IIIa1), 91/180-1 (N30 IIIa2), and 91/180-2 (N36 IIIc1) was administered to 50 Wistar rats/sex/dose in the diet for 24 months at dose levels of 0, 200, 800, 8000, or 16,000 ppm (mean compound intake in males was 0, 9, 36, 375, and 770 mg/kg/day and for females was 0, 12, 47, 497, and 1046 mg/kg/day, respectively).

Clinical observations and mortality were not affected by treatment in either sex of rats. Body weights and body weight gains were decreased relative to controls in 8000 and 16,000 ppm females throughout the study (3.3-16.6% and 11.0-25.8%, respectively; $p \leq 0.05$ or 0.01). The body weights and body weight gains of 8000 and 16,000 ppm males were decreased by 1.7-10.3% and 1.4-14.1%, respectively ($p \leq 0.05$ or 0.01 throughout most or all of the study). The body weight/gain deviations worsened with dose, but only slightly with time. The food consumption and efficiency were similar in control and treated groups. The differential blood count and leukocyte and erythrocyte morphology were not affected by treatment. There were no treatment-related organ weight changes: the $\leq$ 15% increase in the relative brain or liver weight were an artifact of decreased body weights; the $\leq$ 7.5% decrease in absolute kidney weight lacked pathological correlates.

The LOAEL for both male and female rats is 8000 ppm (about 375 mg/kg/day in males and 497 mg/kg/day in females). For males, this is based on the minor decrease in body weight and body weight gain and on the increase in gross and microscopic liver (and biliary) lesions. In females, the LOAEL is based on the lowered body weights and weight gains and on the increased incidence of liver masses. The NOAEL for both sexes is 800 ppm, corresponding to about 36 mg/kg/day in males and 47 mg/kg/day in females.

This study is classified as acceptable, and satisfies the guideline requirement for an oncogenicity study (83-2a) in rats.

83-2b Carcinogenicity [feeding] - Mouse

In a carcinogenicity toxicity study (MRID 43864250), Reg. No. 242 009 (kresoxim-methyl) (96.6% a.i. (Lot #N 30) used for first 28 weeks, and 93.7% a.i. (Lot #N 36) used for remainder of the study) was administered to 50 C57BL/6N CrlBR mice/sex/dose in the diet at dose levels of 0, 400, 2000, and 8000 ppm for 18 months (approximate doses for males of 0, 60, 304, and 1305 mg/kg/day and for females of 0, 81, 400, and 1662 mg/kg/day). An additional 10 animals/sex/dose were treated for 12 months in a satellite study.

Body weight gain was decreased in females following 18 months of treatment at 2000 ppm (22%, $p \leq 0.05$) and in both sexes at 8000 ppm (21%, $p \leq 0.05$; and 39%, $p \leq 0.01$, for males and females, respectively). The incidence of kidney papillary necrosis was increased in females at 8000 ppm (2/50 in control, 13/50 at 8000 ppm, $P \leq 0.01$). Liver amyloidosis was increased in severity and incidence in both sexes at 8000 ppm, but the increased incidence was not statistically significant in males (13/50, control males; 20/50, 8000 ppm males; 6/50, control females; 16/50, 8000 ppm females, $p \leq 0.05$). Amyloidosis was also increased in the adrenal cortex of high dose males compared to the controls (23/50, controls; 34/50, 8000 ppm, $p \leq 0.05$). No compound-related effects were seen in clinical signs, mortality, or hematology.

The LOAEL is 2000 ppm (400 mg/kg/day)for females, based on decreased weight gain, and 8000 ppm (1305 mg/kg/day)for males, based on decreased weight gain, and liver amyloidosis. The NOAEL is 400 ppm (81 mg/kg/day) for females and 2000 ppm (304 mg/kg/day) for males.

This carcinogenicity study in the mouse is acceptable, and does satisfy the guideline requirement for a carcinogenicity study (83-2*b*)in mice.

V. COMMITTEE'S ISSUES FOR CONSIDERATION

The Cancer Review Assessment Committee is asked to review the database on kresoxim-methyl and render decisions on:

1. The carcinogenicity of the compound.

2. Whether the submitted mode of action studies clearly demonstrate how liver tumors are formed in rats.

3. Implication of the entire weight of evidence on the classification of kresoxim-methyl as a carcinogen, and its impact on human risk..

Attachments

Attachement No. 1	Chronic Oral Feeding Study–Rat MRID 43864247 (DER)
Attachement No. 2	Oncogenicity Feeding Study–Rat MRID 43864249 (DER)
Attachement No. 3	Oncogenicity Feeding Study–Mouse MRID 43864250 (DER)
Attachment No. 4	Kresoxim-methyl Qantitative Risk Assessment (Q_1*) (Memo)
Attachment No. 5	Kresoxim-methyl Review of Data Submitted in Support of Mechanism of Action of Rat Liver Tumor Induction. (Memo)
Attachment No. 6	Metabolism–Rat *in vitro*; special non-guideline study MRID 44421001 (DER)
Attachment No. 7	S-Phase Response Study (Dietary–Rat) non-guideline cell proliferation study MRID 44380201 (DER)
Attachment No. 8	S-Phase Response Study (Dietary–Rat) non-guideline cell proliferation study MRID 44392001 (DER)